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SUBJECT OF INVESTIGATION

(6) The explanation of pathogenesis of JBI virus and the establishment of an attenuated strain of JBI virus completely avirulent to man.

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FIRST QUARTERLY REPORT OF THE RESULTS OBTAINED BY THE
RESEARCH UNDER CONTRACT NO. DA-92-557-FEC-35805

Title: The explanation of pathogenesis of JBE virus and the establishment of an attenuated strain of JBE virus completely avirulent to man.

Application of the fluorescent antibody technique to follow viral development within cultivated hamster kidney cells.

In the previous final report, it was demonstrated that the virulent types of JBE virus strains to adult mice were classified into 3 groups, L^+I^+ , L^-I^+ and L^-I^- . Viruses of the former 2 types were distinct from the other with such a property as to multiply outside of brain of adult mice when inoculated peripherally. However, the organ or the tissue of the animal which participate in the primary virus multiplication has been left obscure. Though the previous investigators suggested the involvement of some tissues by the results of the infectivity titration in the viremic phase, the evidences were insufficient due to infective titers of the suspected tissues which were not high enough to deny possible contamination with the infective virus derived from viremic blood.

The currently used fluorescent antibody technique were undoubtedly useful to trail virus growth in tissues and were thought to be worth to apply to solve the present problem. Prior to apply this technique to organs, preliminary study was needed in simpler system employing cultivated cells in vitro. Hamster kidney cells were selected as a susceptible host to JBE virus. Undirect method of fluorescent antibody technique was employed by the matter of convenience.

JBE immune serum was prepared from rabbits immunized with suspension of JBE infected chick embryos. Anti-rabbit γ -globulin serum was prepared from goats and was conjugated with fluorescein isocyanate. Conjugated serum was treated by dried mouse liver powder to absorb non-specific staining materials. Primarily grown hamster kidney cells in square bottles were transferred onto cover slips kept in Petri dishes, and incubated in CO_2 adjusted incubator for 2 days. Cells on cover slips were then placed into square tubes and incubated overnight.

Next day, nine tenth ml of maintenance medium was added after washing cells once and 0.1 ml of virus suspension was inoculated per tube. The multiplicity of infection was high enough (approx. 5:1) to render most of cells infected simultaneously. After the inoculation, cover slips were taken out of 2 infected and one non-infected tubes, washed, fixed by dried acetone for 10 minute and stored in a deep freezer at 2, 6, 10, 15, 24 and 32 hours. Onto fixed cells, 1:20 diluted JBE immune serum (20 HI units for 8 units of HA antigen) was mounted for 30 min, washed then applied with conjugated serum for another 30 min. For the microscopic observation, Reichert's ~~micro~~ apparatus was employed.

Some of pictures taken in this experiment are attached in this report. As shown in Fig. 1 and 2, control cells were stained by fluorescent isocyanate faint and diffuse both in cytoplasm and in nucleus. It was obvious that some non-specific or non-viral staining was unavoidable in our experimental condition. However, it should be pointed out that there was no difference in the illumination of cytoplasm and nucleus. At 10 hours after the inoculation, marked illuminated boundary was noticed around the nucleus in some cells (Fig.3). In contrast to the above, the nucleus appeared as a dark spot. At 15 hours, bright area expanded in cytoplasm from perinuclear site (Fig.4). A sort of bright inclusion isolated from the nucleus which is characteristic to some virus infection was not visible. Illuminated area was diffuse and consisted of fine granules. After 24 hours of incubation, whole part of cytoplasm became illuminated in most of cells as indicated in Fig.5 and 6. The nuclei were still remained unstained. At this period, some cells began to show degenerative changes, i.e. projection, shrinkage and leakage of cytoplasm. By more 8 hours, most of the cells were affected with more or less cytopathic effect. Viral infectivity was measured with culture fluid at each time when cells were examined with fluorescent antibody. Abrupt titer increase was encountered 24 hours after the inoculation.

As a whole, the following facts may be implicated by the present experiment.

1. Viral antigen are produced in cytoplasm and not in the nucleus of the cell.
2. The loci to synthesize viral antigen are scattered diffusely in the cytoplasm.
3. The nucleus may participate in viral growth so as to give a specific information to antigen-producing system within the cytoplasm.

We should remind that these conclusion came from a strain,

Nakayama-NIH of JBE virus and hamster kidney cell system. In order to get a general view concerning JBE virus reproduction in animal cells, similar experiments are now attempted employing various cell-virus system.



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Explanation of plates

- Fig. 1. Uninfected control of hamster kidney cells.
350 x.
- Fig. 2. Uninfected control of hamster kidney cells.
900 x.
- Fig. 3. Infected hamster kidney cells, 10 hours after
the inoculation. 900 x.
- Fig. 4. Infected hamster kidney cells, 15 hours after
the inoculation. 900 x.
- Fig. 5. Infected hamster kidney cells, 24 hours after
the inoculation. 350 x.
- Fig. 6. The same to Fig. 5. 900 x.



